regulators of the transcriptional signature of wound response in breast cancer, and then experimentally validated their hypotheses. This is an exciting achievement in that a transcriptional signature comprising more than 500 genes has been reduced to an interplay between two genes. Given the increasing popularity of gene expression and DNA copy number analyses, the SLAMS method could easily be tested on other data sets to identify candidate regulatory genes.

A few limitations of the approach should be noted. First, the assumption that the dysregulation of a putative regulator's activity is associated with a change in its DNA copy number only applies to a subset of genetic regulators of cancer. Second, the regulator is assumed to be represented on the chip. Although high-density arrays do contain the vast majority of human genes, the regulator may not be present. Third, other types of regulators, such as microRNAs, alternatively spliced products and epigenetic modifications, would be missed by the SLAMS strategy. Fourth, another potential confounder is that some genes in a signature could appear regulated simply because they are part of the regulator's amplicon and not because they are controlled by the regulator. Alternatively, a group of genes in a cluster could point to a candidate regulator erroneously if they are clustered simply because of linkage disequilibrium. Finally, because the authors appear to have benefited from having the well-known oncogene MYC among their candidate regulators, the general applicability of this experimental approach in diverse biological scenarios should be evaluated in less familiar contexts.

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No longer lost in translation

Barry S Cooperman & Yale E Goldman

Two *in vivo* real-time single molecule assays demonstrate that stochastic gene expression results in punctuated bursts of protein synthesis.

Recent advances in fluorescent reporters and microscopy have made it possible to determine the location of individual molecules in live cells and to follow discrete events in real time[1–3]. In two contemporaneous papers published in *Science* (Yu *et al.*[4]) and *Nature* (Cai *et al.*[5]), Xie and coworkers recently described very different approaches to real-time observation of the birth of single protein molecules in *Escherichia coli* cells.

Molecules involved in gene expression, for example, DNA, specific mRNAs and transcriptional and translational regulators, are often present in cells at minute concentrations. The randomness of both the interactions and conformational transitions of these rare molecules leads to fluctuations in the content of a given protein among otherwise identical cells[6–9]. This 'gene noise' limits the precision of expression levels in cellular regulatory networks, but it may also be advantageous in development, adaptation and evolution by facilitating sampling of nearby alternative states that may improve cell function[8]. Theoretical[6] and experimental[7] studies have shown that such fluctuations are inevitable at the low copy numbers of regulators and the rates of cellular processes. Gene noise and its dynamic characteristics have been detected from variations of mRNA and protein expression among cells[6–9], but these ensemble measurements give limited information about the fundamental source of the fluctuations: the synthesis of each individual protein from a particular mRNA.

Novel technologies that make it possible to detect the location, orientation and dynamics of individual fluorophores *in vitro*[10] or within a cell[1–3] have led to dramatic advances in understanding macromolecular folding, energy transduction by molecular motors and the accuracy of nucleic acid–processing enzymes. Single-molecule biophysics might be considered the ultimate in low-throughput bioassays, but by directly monitoring individual reaction pathways, it can reveal important features of dynamic processes that are obscured in ensemble averages. Rare states, thermal fluctuations, reversals and heterogeneities are often difficult to detect in a population, but they are the rule for the chaotic environment of an individual molecule.

In the particular case of protein synthesis, fluctuations in the appearance and lifetimes of biomacromolecules, and thus their number, derive from transcription and translation of the genome, as well as from degradation mechanisms. Yu *et al.* and Cai *et al.* provide new insights into how these processes are coupled, by measuring the expression in *E. coli* of individual proteins under the control of the *lac* promoter, with very low copy numbers resulting from repression in the absence of added inducer, and in the presence of glucose, a catabolic suppressor.

Yu *et al.* monitor the expression of single molecules of a yellow fluorescent protein variant, Venus[11], chosen for the rapid maturation of its intrinsic fluorophore after expression. Even with Venus, however, the rate-limiting step for detecting fluorescence is apparently this maturation, rather than synthesis or folding of the protein. Nevertheless, the timing of Venus synthesis can be deduced by the temporal correlation among separate spikes of fluorescence. Imaging a single Venus molecule in the cytoplasm is difficult because diffusion spreads its fluorescence signal throughout the cytoplasm during image acquisition, reducing the intensity below that of cellular autofluorescence. To overcome this problem, Yu *et al.* fused Venus to a membrane protein, Tsr. Localization of the resulting Tsr-Venus fusion product to the cell membrane slows its diffusion enough to permit single-molecule detection (**Fig. 1a**).

Cai *et al.* use a microfluidic-based assay to quantify expression of β-galactosidase (β-gal) tetramers. Enzymatic activity is measured as an increase in fluorescence upon release of fluorescein from a fluorescein-galactoside substrate, hydrolyzed by β-gal within single cells, each of which is confined to a sealed 100-pl chamber. Such chambers not only serve to isolate one cell from another, but also trap all of the fluorescein formed, which is rapidly pumped out of the cell (**Fig. 1b**). The sensitivity of the assay permits quantification down to one β-gal tetramer per cell. Cai *et al.* have extended this approach to yeast and mammalian cells, demonstrating its versatility.

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Both approaches demonstrate that protein production occurs in bursts, with each burst originating from a stochastically transcribed single mRNA molecule. The bursts occur randomly, uncorrelated in time with each other, and correlate only weakly with the cell cycle. The average time between subsequent bursts is comparable to the *in vitro* dissociation time of the *lac* repressor from the *lac* operator, leading to the intriguing suggestion that each burst is triggered by spontaneous dissociation of the repressor, rather than, for example, clearing of regulators from the DNA as the replisome passes through the genome. The number of molecules per burst displays an exponential distribution, most likely reflecting kinetic competition between RNase E, for degradation of the mRNA, and a ribosome, for the translation initiation site at its 5′ end (**Fig. 1**). This point could be established with greater certainty if the experiments were repeated in cells having reduced RNase E activity or mutated Shine-Delgarno sequences. For reasons that are not made clear, the average number of bursts per cell cycle (0.1–1.2) and the number of peptide chains synthesized per burst (4–20) vary considerably between the experiments in the two papers. It is worth pointing out that quantification of Venus molecules per burst of expression uses a bleaching pulse to avoid accumulation, so that accurate counting requires the plausible, but unproven, assumption that intermediates in the dye maturation process[12] are not photo-inactivated.

Xie and coworkers have introduced several powerful methods for studying the stochastic nature of protein expression, permitting salient conclusions to be reached concerning the origin and dynamics of fluctuations in the activity of the lac operon. Their results clarify the origins of fluctuations in gene expression and open up the possibility that single molecule approaches will be broadly useful for system-wide characterization of gene expression, at least for low copy number macromolecules. By illuminating specific sources of gene noise, their methods should clarify how cells adapt and develop under the chaotic and unpredictable conditions they face. Further, these methods, in concert with those developed for monitoring individual mRNAs[3,9], should be applicable to unraveling the complexities of gene networks and elucidating mechanisms of pathological gene expression. Whether they can be extended to conditions where gene expression is induced, rather than suppressed, and whether comparable behavior is observed, remains to be demonstrated.

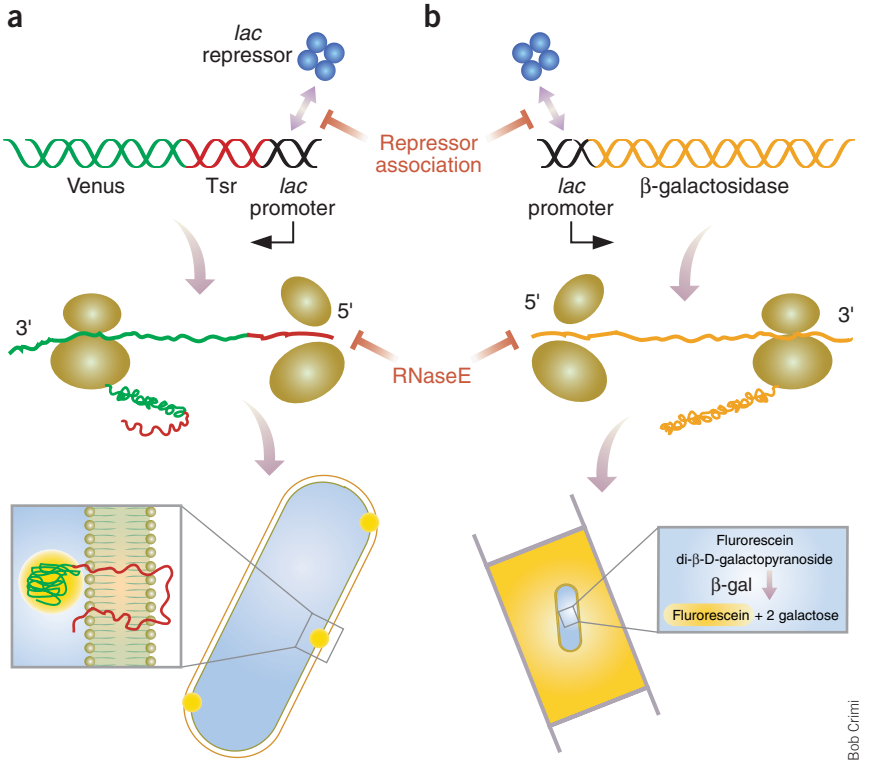

Figure 1 Two approaches to monitor fluorescent bursts of protein synthesis in *E. coli*. Both use the *lac* promoter under conditions that support low levels of transcription. The observation that the average time between bursts of protein accumulation is comparable to the average time taken for the *lac* repressor to dissociate from the *lac* operator suggests that each burst is triggered by spontaneous dissociation of the repressor from its DNA binding site. Competition between RNase E and a newly initiating ribosome for the 5′ end of mRNA is probably a major determinant of the number of protein molecules synthesized per burst. (**a**) The limitations posed by rapid diffusion of the fluorescent protein Venus when it is expressed in the cytoplasm are circumvented by fusing it to a membrane-targeting peptide, Tsr. Reduced diffusion of membrane-anchored Tsr-Venus permits detection of individual proteins. (**b**) Entrapping individual cells in a 100-pl volume in a microfluidic chamber ensures that the fluorescence derived from β-gal activity accurately reflects the bursts of translation.

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